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荧光比率法检测过氧亚硝酸根阴离子的研究以及 CdTe 量子点-米托蒽醌复合物的形成及其应用研究

Study on the Ratiometric Fluorescence for the Detection of Peroxynitrite and Formation of CdTe Quantum Dots - Mitoxantrone Conjugate and Its Application

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Study on the Ratiometric Fluorescence for the Detection of Peroxynitrite and Formation of CdTe Quantum Dots - Mitoxantrone Conjugate and Its Application

A Dissertation Presented

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摘　要

本论文研究内容分为两部分。第一部分为荧光比率法表征过氧亚硝酸根阴离子（$ONOO^-$）的方法研究，及基于此建立抗癌药物米托蒽醌抑制二氧化氮自由基作用评价的比率荧光法研究；第二部分为 CdTe 量子点-米托蒽醌复合物的形成及应用于核酸检测的初步研究。第二部分内容是基于课题组现行研究课题的新的发展方向，为实验中观察到的现象的拓展。

第一部分第一章首先简要介绍了活性氧自由基的定义及研究意义；综述了 $ONOO^-$ 的形成、分解、性质及其表征方法；系统阐述了荧光比率法的检测原理和应用的研究进展，并在此基础上提出了本论文第一部分的研究设想，引用参考文献 160 篇。

第一部分第二章对荧光比率法检测过氧亚硝酸根阴离子的方法进行了研究，该章共分为三节。

第一节：基于铽络合物发光的荧光比率法检测 $ONOO^-$ 的研究。$ONOO^-$ 在弱酸性介质中易质子化生成其共轭酸（ONOOH），ONOOH 均裂生成•OH。•OH 作用于对苯二甲酸发生芳环羟基化反应，生成强荧光物质邻羟基对苯二甲酸。对苯二甲酸具有敏化铽发光的特性，羟基化导致其敏化活性消失。因此，在对苯二甲酸–铽敏化发光体系中加入 $ONOO^-$，将观察到铽的特征发射受到抑制，同时伴随着邻羟基对苯二甲酸的特征发射峰的出现的现象，且随着 $ONOO^-$ 浓度的加大，430 nm 处的邻羟基对苯二甲酸特征发射与 545 nm 处的铽特征发射之比值呈增大的趋势，从而可以利用这两个峰的强度变化比来表征 $ONOO^-$。

第二节：基于芘激基缔合物形成的荧光比率法检测 $ONOO^-$ 的初步研究。该项研究的基本思路是发展基于芘的二聚体–单体平衡的、以 $ONOO^-$ 为目标分子的比率型荧光探针，即以芘分子标记酪胺，当酪胺与目标分子 $ONOO^-$ 作用时易发生二聚，酪胺的二聚，拉近了分别标记于两个酪胺分子上的芘分子，两个芘分子由于距离接近而出现激基缔合物发射，与之同时，伴随着单体发射的荧光峰减弱，激基缔合物态与单体态发射的荧光强度之比 I_E/I_M 应与目标分子 $ONOO^-$ 的浓度相关，以此为基础发展建立基于荧光比率原理的 $ONOO^-$ 荧光分析法。这部分实验只进行了初步的探索，尚未达到预期的效果。

第三节：基于酪氨酸二聚的同步荧光法比率法检测 $ONOO^-$ 的研究。酪氨酸与目标

分子 $ONOO^-$作用时，如果控制酪氨酸的浓度远大于 $ONOO^-$，可得到单体发射恒定不变而二聚体的发射则随目标分子的浓度的增大而增强的同步发射光谱特征。采用同步荧光扫描，以酪氨酸自身的荧光作内参比，二聚酪氨酸的荧光强度与酪氨酸自身的荧光强度的比值与 $ONOO^-$的浓度在一定范围内呈线性相关，且能不受仪器测试参数改变影响，从而建立了一种基于内参比的同步荧光比率检测 $ONOO^-$的方法。

第一部分第三章是在第二章第三节的实验结果的基础上，建立了用于评价抗癌药米托蒽醌清除二氧化氮自由基效力的荧光法。二氧化碳催化 $ONOO^-$产生$\cdot NO_2$，$\cdot NO_2$诱导强荧光酪氨酸二聚体形成。米托蒽醌具有抗氧化活性，当体系中引入米托蒽醌时，米托蒽醌与酪氨酸竞争$\cdot NO_2$，抑制酪氨酸二聚，表现为二聚体荧光强度降低。据此建立了一种间接表征米托蒽醌抗氧化效力的方法。

第一部分第四章是对第一部分的研究工作的总结，指出工作的不足，并对研究工作的前景提出了设想。

第二部分第一章首先简要介绍了量子点及其复合物研究意义，并在此基础上提出了研究的设想。然后根据文献合成了水溶性的巯基乙酸包裹的 CdTe 量子点，进而依据静电作用原理，制备了量子点- 米托蒽醌（QD-MXT）复合物。与 CdTe 量子点具有强发光特性形成强烈反差的是该复合物中量子点的荧光几乎完全猝灭，为一非荧光复合物。当 QD-MXT 复合物作用于 DNA 后，由于 DNA 与米托蒽醌发生分子间作用，导致量子点荧光回复。基于对光谱性质的分析, 就复合物发光猝灭机制与 DNA 响应机制进行了探讨。对应用 QD-MXT 复合物表征 DNA 进行了初步研究。

第二部分第二章是对第二部分的研究工作的总结，指出工作的不足，并对研究工作的前景提出了设想。

关键词：过氧亚硝酸根阴离子；荧光比率法；酪氨酸；量子点；复合物；DNA

Abstract

The dissertation consists of two parts. The first part is focused on the study on the analytical methodology based on ratiometric fluorescence for the detection of peroxynitrite($ONOO^-$) and evaluation of the scavenging effect of mitoxantrone to $\cdot NO_2$. The second one presents some preliminary experimental results related to CdTe Quantum Dots - mitoxantrone conjugate and its application in determination of nucleic acids.

In chapter 1 of part one, a brief review on the biological active species, $ONOO^-$, is given, concerning its formation, decomposition, reactivity, and its biological significancy, as well as the methods for the determination of peroxynitrite that have been developmented and used in analytical science and bioscience. A systematic review on the theory and application of ratiometric fluorescence are also presented. 160 references are cited. The research plan for part one of the present dissertation is given.

In chapter 2 of part one, the results of a serie studies on the analytical methodology based on ratiometric fluorescence for the detection of peroxynitrite are presented. This chapter contains three sections.

In section one, a study on ratiometric determination of $ONOO^-$ using Terbium(Ⅲ) complex as a fluorescent probe is presented. $ONOO^-$ decomposes to free radical of nitrite ($\bullet NO_2$) and hydroxyl radical (•OH) on protonation. •OH would react rapidly with terephthalic acid and transfer the latter to a high fluorescence compound, 2-hydroxyl-terephthalic acid. Terephthalic acid would sensitize the luminescence of Tb(Ⅲ) through a intramolecular energy transfer manner on coordinating with Tb(Ⅲ). The formation of 2-hydroxyl-terephthalic acid could greatly attenuate the sensitization of Tb(III)'s luminescence, thus, an increase in the fluorescence intensity of 2-hydroxyl- terephthalic acid at 430 nm and a decrease of the fluorescence intensity of Tb(III) at 545 nm would be observded in the presence of $ONOO^-$. The intensity ratio, F_{430}/F_{545}, was proportional to the concentration of the hydroxyl radical, and thus to the concentration of peroxynitrite.

In section two, a ratiometric fluorimetric method for detection of peroxynitrite based

on the formation of excimer of pyrene was explored. Tyramine was labeled with pyrene. When dityramine was formed due to reaction tyramine with peroxynitrite, excimer of pyrene would form as a result of the short distance between the two pyrene molecules. The increase in fluorescence of excimer would be observed and, at the same time, the decrease in fluorescence of monomer of pyrene would be measured. The ratio of the fluorescence intensity of monomer (I_M) versus the fluorescence intensity of excimer (I_E) would have a good response to peroxynitrite. This hypothesis, however, the expected results have not been gotten yet.

In section three, a ratiometric synchronous fluorimetric method for detection of peroxynitrite was developed based on the formation of dityrosine. Tyrosine acted as inner reference. The ratio of the fluorescence intensity of dityrosine versus the fluorescence intensity of tyrosine was not affected by instrument parameter and had a good response to peroxynitrite by synchronous fluorescence spectrometry.

In chapter 3 of part one, based on the result obtained in section three of chapter 2, a new method was established for evaluation of the scavenging effect of mitoxantrone to $\cdot NO_2$ by fluorescence spectrophotometry. The transformation of tyrosine to dityrosine is mediated by $\cdot NO_2$ produced from $ONOO^-$ and and catalized by CO_2. The production of dityrosine was attenuated in the presence of mitoxantrone due to the scavenging action of mitoxantrone on $\cdot NO_2$ and, thus the fluorescence produced by dityrosine was reduced. The change of fluorescence had a good response to mitoxantrone.

In chapter 4 of part one, the research work of part one was summarized, and the expectation and limitation of this research were given.

In chapter 1 of part two, the brief introduction concerning quantum dots and its conjugate are given. Water-soluble CdTe quantum dots were prepared and conjugated with mitoxantrone through electrostatic interaction. Upon this reaction, the fluorescence of QD was quenched. When DNA was added, the fluorescence of QD was increased. A possible mechanism of the interaction was proposed. Thus, a novel approach was developed for detection of DNA.

In chapter 2 of part two, the research work of part one was summarized, and the